# NEOTROPICAL MONOGENEA. 7. PARASITES OF THE PIRARUCU, ARAPAIMA GIGAS (CUVIER), WITH DESCRIPTIONS OF TWO NEW SPECIES AND REDESCRIPTION OF DAWESTREMA CYCLOANCISTRIUM PRICE AND NOWLIN, 1967 (DACTYLOGYRIDAE: ANCYROCEPHALINAE)

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Abstract. – Dawestrema cycloancistrium Price and Nowlin, 1967, D. cycloancistrioides n. sp., and D. punctatum n. sp. are reported and described from the pirarucu, Arapaima gigas (Cuvier), collected from the Solimões River near Manaus, Amazonas, Brazil. An emended generic diagnosis of Dawestrema Price and Nowlin, 1967, is presented incorporating new information on internal anatomy and structure of the copulatory complex and haptor. A summary of the parasitic helminths reported from A. gigas is provided.

The pirarucu, Arapaima gigas (Cuvier), Osteoglossidae, inhabits the Amazon River drainage, the western Orinoco and the Rupununi and Essequibo river systems of the Guianas, and is thus limited in its distribution to parts of northern South America. This fish is of considerable economic importance and is regarded as a food fish of the highest quality.

Studies on the parasites of the pirarucu were begun during the early nineteenth century with the description of larval *Gnathostoma gracilis* by Diesing (1838). Including that record, a total of 14 helminth species have been reported from this fish. Baylis (1927) listed the following (names appearing below are as given by Vicente and Pinto 1981, Rego et al. 1974, and Noronha 1981): Nematoda— *Goezia spinulosa* (Diesing, 1839), *Terranova serrata* (Drasche, 1884),\* *Camallanus tridentatus* (Drasche, 1884), and *Gnathostoma gracilis* (Diesing, 1838); Acanthocephala—*Polyacanthorhynchus macrorhynchus* (Diesing, 1856); and Cestodaria—*Schizochoerus liguloideus* (Diesing, 1850), and *Nesolecithus janickii* Poche, 1922. Baylis (1927) also described the nematode, *Philometra senticosa* (*Nilonema senticosa* in Vicente and Pinto 1981), from the swim-bladder of the fish. Machado Filho (1947) reported *Polyacanthorhynchus rhopalorhynchus* (Diesing, 1851) and *P. macrorhynchus* (Diesing, 1856) from *A. gigas*; and Travassos (1960) reported the nematode, *Rumai rumai*, as a new species from the host's body cavity. Prudhoe (1960) described *Caballerotrema brasiliense* (Trematoda)

<sup>\*</sup> According to Baylis (1927), *Porrocaecum draschei* (Stossich, 1896) is a synonym of this species since *Ascaris serrata* "was very briefly described by v. Drasche (1884), who had before him only a single male specimen. Stossich (1896), who changed the name of the species to *A*[scaris]. draschei (apparently on the ground of the previous existence of *Ancyracanthus serratus* Wright, 1879, which he believed to be an *Ascaris*, but which is probably to be referred to *Cystidicola*), added nothing to the description."

from the intestine of the host. This species was redescribed, and *C. arapaimense* proposed as a new species from pirarucu by Thatcher (1980). *Himasthla piscicola*, described by Stunkard (1960) from the intestine of the host, is likely a synonym of one of the *Caballerotrema* species. The only monogene previously reported from *A. gigas* is *Dawestrema cycloancistrium* Price and Nowlin, 1967. The present study adds two new species to the parasite fauna of this host and includes a redescription of *D. cycloancistrium*.

### Materials and Methods

The host was collected from the Solimões River near Manaus, Amazonas, Brazil, on 15 April 1983. Fish gills were placed in finger bowls and covered with a 1:4000 formalin solution. After one-half hour, the gills were agitated in this liquid and then removed from the bowl. The helminths were allowed to settle to the bottom and were subsequently removed with the aid of a small probe and dissecting microscope. They were immediately fixed and stored in AFA. Some were mounted unstained in Gray and Wess' medium for study of sclerotized structures. Other specimens stained with Semichon's carmalum or Gomori's trichrome were used to determine internal features. Measurements were taken only on specimens collected during the present study; all, in micrometers, were made according to the procedures of Mizelle and Klucka (1953) except that the cirrus measurement is the diameter of the largest ring of the coil. Numbering of hook pairs follows that proposed by Mizelle (1936). Illustrations were prepared with the aid of a camera lucida or microprojecter. Type-specimens are deposited in the collections of the Instituto Nacional de Pesquisas da Amazônia (INPA), the U.S. National Museum Helminthological Collection (USNM), and the University of Nebraska State Museum (UNSM) as indicated below.

Species of many genera of Monogenea from the Neotropical Region are characterized by a cirrus comprising a variably developed base from which a coiled shaft arises. The coil of the shaft may consist of less than one complete ring to many rings. While the direction of the coil has not been specifically addressed in previous studies on Neotropical forms, a counterclockwise or clockwise coil may be shown to have diagnostic value at the specific and/or generic level as understanding of the Monogenea from this region develops. Thus, the coil direction is determined by viewing the cirrus in ventral view. If the cirrus shaft is directed in a clockwise direction from the base to the ventral tip of the shaft, the rings are defined to have a clockwise direction, and conversely so, counterclockwise. In the present study, all species of *Dawestrema* were found to have counterclockwise rings, and this character is incorporated into the emended diagnosis of the genus.

#### Dawestrema Price and Nowlin, 1967

*Emended diagnosis.*—Dactylogyridae, Ancyrocephalinae. Body elongate, divisible into cephalic region, trunk, peduncle, and haptor. Tegument thin, smooth. Head organs, cephalic glands present. Four eyes. Mouth subterminal; pharynx muscular, glandular; esophagus elongate; intestinal crura 2, confluent in posterior trunk, lacking diverticulae. Gonads intercecal, slightly overlapping or tandem; testis postovarian. Vagina sinistroventral; seminal receptacle overlapping or lying immediately anterior to ovary along body midline; uterus delicate; genital pore

midventral. Vitellaria well developed as 2 bilateral bands coextensive with gut; eggs with terminal filament(s). Vas deferens looping left intestinal crus; 2 seminal vesicles, simple dilations of sperm duct; prostatic reservior present; cirrus comprising a coil of one to many counterclockwise rings; accessory piece articulated to cirral base, proximal part lying within cirrus coil, terminal portion serving as cirrus guide. Haptor with 2 pairs of anchors (dorsal and ventral), dorsal and ventral bars (nonarticulated), 7 pairs of hooks. Hook pairs 1, 2, 3, 4, 6, 7 arranged concentrically around haptor usually anterior to anchors. Ventral bar with medial anterior projection arising from posterior or dorsal margin of bar. Parasitic on gills of freshwater fishes of the Osteoglossidae.

Type-species, host, and locality. – Dawestrema cycloancistrium Price and Nowlin, 1967, from the gills of Arapaima gigas (Cuvier), from Amazon River and tributaries, Brazil.

Other species. – Dawestrema cycloancistrioides n. sp., D. punctatum n. sp., both from the gills of Arapaima gigas, Solimões River near Manaus, Amazonas, Brazil.

# Dawestrema cycloancistrium Price and Nowlin, 1967 Figs. 1-11

Specimens studied.—Holotype, USNM 62985; voucher specimens, INPA 00000, USNM 78224, UNSM 22092.

Description (based on 22 specimens and holotype).-Body graciliform; length 2180 (1618-2552), greatest width 112 (83-138) near level of vagina or cephalic region. Cephalic margin rounded, lobes poorly developed; head organs comprising loosely associated cephalic gland ducts; postpharyngeal bilateral groups of unicellular cephalic glands well developed. Each eye with lens; members of posterior pair of eyes larger, farther apart than those of anterior pair; eye granules small, variable in shape; accessory granules absent. Pharynx spherical, 62 (45-72) in diameter; gut confluent apparently at several locations posterior to testis. Peduncle elongate, with pair of conspicuous glands; haptor bulbous, with anchors situated on posterior lobe; haptor 156 wide, 129 (86-159) long. Anchors similar; ventral anchor robust, with elongate straight point, heavy base, ornate deep root, superficial root with conspicuous saddle-like fold; ventral anchor 35 (33-37) long, base 25 (23-28) wide. Dorsal anchor with curved point and shaft, fold of superficial root weakly developed; dorsal anchor 51 (47-54) long, base 35 (29-43) wide. Anchor filament simple, double, conspicuous. Ventral bar plate-like, with anterior medial projection arising near posterior margin; bar 25 (21-29) long. Dorsal bar with globose ends, heavy ridge along posterior margin; bar 38 (28-43) long. Hook distribution typical with ring of hooks well anterior to anchors; hook 16 (13-18) long; pairs 1, 2, 3, 4, 6, 7 similar; each with depressed thumb, shank with small terminal enlargement, point recurved, FH loop <sup>4</sup>/<sub>5</sub> shank length; pair 5 delicate, with small enlargement of proximal end of shank, FH loop 1/2 shank length. Testis ovate, postovarian, 29 (21-37) wide, 57 (46-74) long; seminal vesicles fusiform; prostatic reservoir elongate, thin walled, frequently twisted. Cirrus with 5-7 rings, slightly enlarged base, largest ring diameter 42 (37–48); accessory piece terminally enclosing cirrus shaft. Ovary elongate ovate, 28 (24-33) wide, 108 (94-121) long; oviduct short; ootype not observed; uterus (when empty) a delicate duct with terminal expansion, extending to right of midline; genital pore at level of cirrus;

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vagina tubular, proximally coiled, with distal petal-shaped sclerotization protruding from aperture; seminal receptacle pyriform, lying anterior to vagina coil. Vitellaria appearing as hollow tubular longitudinal structures. Egg elongate ovate, with proximal filament; filament exceptionally long; egg 136 (126–147) by 34 (29– 37).

*Remarks.*—Examination of the holotype of *Dawestrema cycloancistrium* and comparison of it with our specimens showed them to be conspecific. The holotype, mounted unstained in glycerine jelly, is apparently contracted as a result of fixation procedures, which explains the significant differences of body shape and size between our specimens and measurements and drawings provided by Price and Nowlin (1967). Further, we determined several erroneous interpretations in the original description concerning the internal anatomy and structure of the sclerotized haptoral armament and copulatory complex. These are evaluated in the discussion below.

## Dawestrema cycloancistrioides, new species Figs. 12–20

*Type-specimens.*—Holotype, INPA 00000; paratypes, INPA 00000, USNM 78222, UNSM 22093.

Description (based on 24 specimens).-Body cylindrical, tapered at extremes; length 1281 (1015–1766), greatest width 92 (73–118) at or posterior to gonads. Two terminal, 2 subterminal cephalic lobes well developed; head organs distinct in each lobe; cephalic glands well developed, comprising prepharyngeal, pharyngeal, and postpharyngeal bilateral paired groups of unicellular glands; bilateral pair also lying immediately anterior to level of vagina. Members of posterior pair of eyes with lenses, larger and farther apart than those of anterior pair; eyespot granules small, variable in size and shape; accessory granules usually restricted to immediate region of eyes. Pharynx spherical, 53 (40-60) in diameter. Peduncle moderately elongate; haptor bulbous, tapered posteriorly with ventral anchors situated on posterior lobe; haptor 83 (60-107) wide, 82 (65-122) long. Anchors dissimilar; ventral anchor robust, with evenly curved point and shaft, exaggerated and truncate superficial root, small deep root; anchor 49 (46-51) long, base width 28 (24-30). Dorsal anchor with delicate, evenly curved point and shaft, vestigial deep root, tapered superficial root; anchor 32 (29-34) long, base width 22 (17-28). Anchor filament simple, double, conspicuous. Ventral bar plate-like, with anterior projection originating from posterior margin of bar; bar 21 (20–23) long. Dorsal bar broadly U-shaped with slightly enlarged ends; bar 28 (26-30) long. Hook pairs 1, 2, 3, 4, 6, 7 distributed around the widest part of the haptoral bulb at level of dorsal anchor bases; hooks 1, 2, 3, 4, 6, 7 similar, each with two-part shank, slightly depressed thumb, tapered shaft and point; hook pair 5 with slender

Figs. 1-11. Dawestrema cycloancistrium: 1, Composite drawing of whole mount (ventral); 2, Copulatory complex; 3, Enlargement of worm at level of reproductive systems (ventral); 4, Vagina; 5, Hook (pair 1); 6, Hook (pair 5); 7, Ventral bar; 8, Dorsal bar; 9, Ventral anchor; 10, Dorsal anchor; 11, Egg. All figures are reproduced to the same scale (30 micrometers) except Figs. 1, 11 (500 micrometers, 100 micrometers, respectively).



Figs. 12–20. Dawestrema cycloancistrioides: 12, Ventral view of holotype; 13, Vagina; 14, Copulatory complex; 15, Ventral bar; 16, Hook (pair 1); 17, Hook (pair 5); 18, Dorsal bar; 19, Dorsal anchor; 20, Ventral anchor. All figures are drawn to the same scale (30 micrometers) except Fig. 12 (200 micrometers).

shank and small proximal enlargement; hook length 19 (17–23). FH loop extending to union of 2 parts of shank. Cirrus a coil of 7–8 rings, with flared base, largest ring diameter 33 (28–37); accessory piece terminally enclosing cirral shaft. Gonads overlapping. Testis dorsoposterior to ovary, elongate ovate, 16 (14–19) wide, 64 (43–85) long; seminal vesicles stout, fusiform, anterior vesicle larger; prostatic reservoir a delicate pyriform sac lying near level of anterior seminal vesicle; prostrate not observed. Ovary spindle-shaped, elongate, 34 (20–47) wide, 77 (74– 79) long; oviduct short; ootype not observed; uterus delicate, extending along ventral midline opening into midventral genital atrium by darkstaining structure apparently functioning as sphincter; vagina comprising a distal funnel with elongate twisted tube, opening into inconspicuous seminal receptacle; vitellaria confluent posteriorly, vitelline commissure lying at level immediately anterior to vaginal coils; egg ovate with proximal short filament, 80 (61–98) by (45–46).

*Remarks.*—*Dawestrema cycloancistrioides* most closely resembles *D. cycloancistrium*, as shown by the comparative morphology of the copulatory complex. The new species differs from *D. cycloancistrium* by having 1) a smaller body size, 2) a vaginal tube without a tight proximal coil, 3) a short anteromedial process of the ventral bar, 4) robust hooks (except pair 5), 5) anchors of a different morphology, and 6) a short proximal egg filament. The specific name, from Greek, indicates the relationship of these two species.

## Dawestrema punctatum, new species Figs. 21-29

*Type-specimens.*—Holotype, INPA 00000; paratypes, USNM 78223, UNSM 22094.

Description (based on 5 specimens).—Body spindle shaped; length 852 (796-923), greatest width 86 (65–114) near midlength or in anterior half. 2 terminal, 2 bilateral cephalic lobes well developed; some specimens with an incipient lobe between major pairs. Head organs well developed, one in each cephalic lobe; cephalic glands not observed. Members of posterior pair of eyes larger, closer together than those of anterior pair, lens usually visible in posterior pair; eye granules subspherical, small, variable in size; accessory granules generally absent (eye granules frequently disassociated in flattened specimens). Pharynx spherical, 40 (37–43) in diameter; esophagus moderate in length; gut apparently normal. Peduncle elongate to moderately long; haptor bulbous, tapered posteriorly, with ventral anchors situated on posterior lobe; haptor 108 (98-119) wide, 89 (75-106) long. Anchors similar, each with well-developed base, roots moderately developed, elongate point with terminal recurved tip, ventral anchor 54 (53-55) long, base width 30 (28-32); dorsal anchor 41 (35-45) long, base width 25 (21-30). Anchor filament simple, double, inconspicuous. Ventral bar subrectangular, with anterior projection arising from dorsal surface, bar 30 (26-33) long; dorsal bar rod-shaped with slightly enlarged ends, bar 36 (32–39) long. Hook pairs 1, 2, 3, 4, 6, 7 lying in ring at level of dorsal anchor bases, similar in shape, 21 (18-24) long, each with slender distal shank, proximal shank enlarged, thumb erect, point recurved; hook pair 5 with slender shank and small proximal enlargement, 13 (12-14) long. FH loop extending to union of 2 parts of shank. Cirrus a coil of about 1<sup>1</sup>/<sub>2</sub> rings, base of cirrus lying ventral in body, with first ring of shaft directed dorsally, termination of shaft curved ventrally, ring diameter 39 (37-41); accessory



Figs. 21–29. Sclerotized parts of *Dawestrema punctatum*: 21, Ventral bar; 22, 23, Dorsal bars; 24, Vagina; 25, Copulatory complex; 26, Hook (pair 1); 27, Ventral anchor; 28, Hook (pair 5); 29, Dorsal anchor. All figures are at the 30 micrometer scale.

piece variable, terminally enclosing cirrus shaft. Gonads overlapping. Testis elongate, dorsoposterior to ovary; loop of vas deferens immediately posterior to vagina; seminal vesicles c-shaped; prostatic reservoir with outer circular muscles and enlarged duct. Limits of ovary not observed; oviduct short; ootype not observed; uterus delicate, frequently containing single egg; genital pore midventral at level of cirrus; vagina a short sclerotized tube flaring proximally and opening simply at left margin; vitellaria generally distributed throughout trunk, commissure anterior to seminal receptacle; egg elongate ovate, with terminal short filament at each pole; egg 132 long, 32–33 wide.

Remarks.—This species differs significantly from both Dawestrema cycloancistrium and D. cycloancistrioides in the comparative morphology of the haptoral armament, copulatory complex, vagina, and egg. However, it is apparently closest to D. cycloancistrioides based on the similar nature of the superficial roots of the anchor bases. The specific name is from Latin (punctata = thorn) and refers to the recurved tips of the anchor points.

### Discussion

Price and Nowlin (1967) proposed *Dawestrema* for monogenes characterized by having a circle of hooks located well anterior to the remainder of the haptoral armament, two prostatic reservoirs, an intercecal vas deferens, and apparently non-confluent intestinal crura. However, our examination of the holotype confirmed that one of our forms was the type-species, *D. cycloancistrium*, and that errors in the original study had been made concerning the structure of the copulatory complex, ventral bar, and internal anatomy which necessitated a redefinition of the genus.

While the hook arrangement in *Dawestrema cycloancistrium* is as originally described, our discovery of how two new species show that the anterior position of the ring of hooks is only a specific trait. In *D. cycloancistrioides* and *D. punctatum*, the hook ring is at the level of the bases of the dorsal pair of anchors. Hook pairs 1 (submedial), 2, 3, and 4 are ventral, each pair situated more laterally, respectively. Pairs 6 and 7 continue the ring on the dorsal surface of the haptor with pair 7 being nearest the midline. In those species in which the hook ring is at the level of the species in which the hook ring is at the level of the bases, pair 7 lies lateral to the anchors. Pair 5 (ventral) lies between the shafts and points of the more posterior ventral anchors.

All known species of *Dawestrema* possess a medial, anteriorly directed projection on the ventral bar. Price and Nowlin (1967) indicate in their fig. 8 that the origin of the process is the anterior bar margin, while in all of our specimens, the process originates from the posterodorsal or posterior bar surfaces.

Availability of three species of *Dawestrema*, some specimens of which were stained for study of internal structure, provided an opportunity to clarify morphologic features of the reproductive and digestive systems. Our findings differed from the original description as follows: (1) the presence of two tandemly arranged seminal vesicles (one described by Price and Nowlin 1967, which probably represents the anterior expanded part of the uterus); (2) a vas deferens looping the left intestinal crus (intercecal in the original description); (3) one prostatic reservoir (two in Price and Nowlin 1967); (4) confluent intestinal crura (Price and Nowlin apparently mistook the hollow nature of the bilateral vitelline bands in *D. cycloancistrium* as intestine); and (5) a testis smaller than ovary.

Lastly, our specimens clearly show that the proximal portion of the accessory piece (connecting piece of Price and Nowlin 1967) is located within the rings of the cirrus coil and not external to them as originally described. This configuration of the copulatory complex, along with observations on specimens in which the terminal portion of the cirral shaft was partially protruded from the genital pore, provides some insight to the functional morphology of this structure during copulation. In specimens with partly protruded cirri, the cirrus base may have the bottom surface facing ventrally, while in others this surface faces dorsally. In addition, the diameter of the cirral rings does not change appreciably in specimens with extruded cirri. Since the terminal part of the accessory piece, serving as a cirrus guide, is apparently fixed in location at the genital pore or within the genital atrium, these observations suggest that protrusion of the cirrus during copulation is not a result of a tightening of the cirrus coil, but rather an unwinding of the cirral shaft with the cirrus base rotating as the shaft is extruded. The twisted nature of the proximal part of the accessory piece, its articulation with the base of the cirrus, and the supposed fixed location of the terminal part of the accessory piece in the genital atrium, suggest that the accessory piece serves as a means of retracting the cirrus shaft much the same as the spring of a carpenter's tape measure. This functional aspect, based on observed structure of fixed specimens, however, is assumed as the mechanism employed, and confirmation will depend on observation of living specimens and/or analysis of the composition of the proximal part of the accessory piece. This is the first time, nonetheless, that insight into mechanical function of these structures has been available.

Price and Nowlin (1967) suggested that the long cirrus shaft of *D. cycloancistrium* provides a survival advantage for copulation over some distance. However, comparison of the structure of the vaginae and the length of the cirral shafts of respective species in the genus would not suggest that this occurs. In *D. cycloancistrium* and *D. cycloancistrioides*, the elongate cirral shaft would be necessary to reach the seminal receptacle through the long, tortuous, and/or coiled vaginal tube, while in *D. punctatum*, the comparatively short cirral tube could effectively reach the seminal receptacle since the vaginal tube is correspondingly short. Similar relationships in cirral and vaginal tube lengths occur in other monogeneans (e.g., *Gonocleithrum aruanae* and *G. cursitans*, see Kritsky and Thatcher 1983).

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Note added in proof: Specimens for deposition in the helminthological collection at INPA were shipped on 31 May 1984 and to this date (11 March 1985) have not been received in Manaus. We assume that they have been lost, at least temporarily, and thus no INPA numbers have been assigned. Lost specimens include the holotypes of *D. cycloancistrioides* and *D. punctatum*, 6 paratypes of *D. cycloancistrioides*, and 6 vouchers of *D. cycloancistrium*.